Debrisoquine Metabolism and Lung Cancer Risk[1]

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Abstract

Previous reports of the association between the debrisoquine metabolic polymorphism and lung cancer risk have been conflicting. We examined the hypothesis that the genetically determined ability to metabolize debrisoquine identifies individuals at increased risk for lung cancer in a study designed to address some of the methodological criticisms of previous studies. A case-control study of 335 incident Caucasian lung cancer patients and 373 controls matched for age, race, sex, and hospital, was conducted at the National Naval Medical Center (Bethesda, MD) and at the Laval Hospital (Sainte-Foy, Quebec, Canada). Debrisoquine metabolic phenotype was determined by debrisoquine administration and analysis of debrisoquine and 4-hydroxydebrisoquine in the subsequent 8-h urine collected. Stratified and logistic regression analyses were used to evaluate the association between extensive or intermediate debrisoquine metabolism and lung cancer risk. We found no increased risk among extensive or intermediate metabolizers (odds ratio, 0.6; 95% confidence interval, 0.3–1.2). The lack of an association was not confounded by control diagnoses, medications used within 1 month of debrisoquine administration, smoking, stage, or histology of lung cancer. No relationship was found among either heavy smokers or light and nonsmokers. Our results do not support the role of debrisoquine metabolism as a marker for lung cancer risk. While the concept that polymorphisms of metabolism may account for differential susceptibility to lung cancer is sound, debrisoquine metabolic phenotype was not associated with lung cancer risk in these data.

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Introduction

Despite 90% of lung cancer being attributable to tobacco use, only a fraction of smokers develop lung cancer (1). This differential susceptibility has prompted investigation of other potential etiological factors. Host susceptibility based on interindividual variation in carcinogen metabolism as measured by differential drug metabolism has been an area of active investigation. The debrisoquine 4-hydroxylase genetic deficiency is one of the most widely studied human drug oxidation defects (2–6). Studies indicate between 3–9% of Caucasians are deficient in this enzyme and are considered poor metabolizers (3, 7–8). Previous studies using either debrisoquine metabolic phenotype or genotype have reported an association of extensive metabolism of debrisoquine with increased lung cancer risk (9–14), although others have found no association (15–19).

Some investigators have speculated that the reported association between extensive debrisoquine metabolism and tobacco-induced lung cancer is a result of activation of a mutagenic compound in tobacco smoke, *NNK*,[4] by *CYP2D6* (20), the cytochrome P-450 gene responsible for debrisoquine metabolism (18–19). Others have suggested that *CYP2D6* is involved in the metabolism of nicotine to cotinine (21).

The evidence linking lung cancer to the debrisoquine metabolic phenotype is equivocal, with several studies in addition to ours finding no excess risk for IM/EM (15–18), and others reporting an excess number of extensive metabolizers among lung cancer cases (9–12). Problems with the laboratory assay and variations in study methodology may be possible explanations for the inconsistencies. Studies have used various phenotyping methods with doses of debrisoquine administered ranging from 1 mg (18) to doses based on body weight (16), while most studies have used 10 mg (9–12, 15, 17). Duration of urine collection has ranged from 4 to 8 h (16), and daytime and overnight phenotyping results have been combined in the same study (10). Others have included prevalent lung cancer cases (10, 17–18), patients who have received chemotherapy and/or radiotherapy (17–18), control subjects selected specifically with a diagnosis of smoking-related lung disease (9–10, 15), controls with other malignancies (10), and lung cancer patients with no concurrent comparison group (18). Others have used healthy volunteers (9, 13, 15, 17) or unmatched hospital controls (16) as the referent group, and therefore not matched to cases on age or sex, and with medication usage and smoking information unavailable for adjustment. In addition, some analyses have grouped racial or ethnic groups despite evidence of differences in debrisoquine

[4] The abbreviations used are: *NNK*, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; IM, intermediate metabolizers; EM, extensive metabolizers; PM, poor metabolizers; GC, gas chromatography; MR, metabolic ratio; OR, odds ratio; CI, confidence interval; COPD, chronic obstructive pulmonary disease.

metabolism (10, 18). Concurrent medication use among case subjects has been dealt with differently including no medication exclusions (15, 18), medication withheld for 9.5 h prior to beginning debrisoquine phenotyping (9), and exclusion of subjects taking specific medications known to be metabolized by *CYP2D6* (10–12, 16–17). Some studies excluded all medication use among control subjects (11, 15). The only published study reporting response rates achieved participation from only 96 of 291 (33%) eligible cases (10). The laboratory assay used for determination of debrisoquine and its metabolite has also varied among studies from electron-capture GC (9–12, 15–16) to GC-mass spectroscopy (18), to gas-liquid chromatography (17). Consequently, previous studies are not all comparable.

We now present our findings on lung cancer risk and debrisoquine metabolism from a case-control study designed to overcome some of the methodological criticisms cited in other studies.

Subjects and Methods

Subjects. A collaborative case-control study of incident lung cancer was conducted at the Laval Hospital, Sainte-Foy, Quebec, Canada and at the National Naval Medical Center, Bethesda, MD from August 1988 to February 1992. The protocol was approved by all participating institutional review boards, and all study subjects gave signed informed consent. All departments evaluating patients with possible lung cancer, including thoracic surgery, pulmonary medicine, radiation oncology, medical oncology, and internal medicine, were regularly contacted to identify all incident cases, and pathology accession logs were reviewed to identify any additional histologically confirmed lung cancers. By enrolling suspected lung cancer cases, we frequently completed metabolic phenotyping before patients were on any medication, and before surgical procedures were performed. All phenotyping was conducted before any therapy for lung cancer. Cases were included in the analysis only after their histological diagnoses of lung cancer were confirmed by pathology review by lung cancer pathologists at each institution.

Control subjects were recruited from the outpatients with scheduled appointments in the Urology and Orthopedic Surgery Clinics at the National Naval Medical Center (Bethesda, MD; hereafter referred to as Bethesda) and from inpatients at Laval Hospital (Ste-Foy, Quebec, Canada; hereafter referred to as Quebec). At Bethesda, individuals were identified from the clinic schedules, screened by telephone prior to their scheduled appointment, and frequency matched to cases by 5-year age group, sex, and race. At Quebec, sex, race, and 5-year age group-matched individuals were identified on the inpatient services, and after consent was obtained from their physicians, they were invited to participate in the study.

A normal baseline blood pressure and a cardiovascular examination were required for participation. Exclusions from study participation were intensive care unit treatment, inability to give informed consent, blood pressure <90/60, severe renal disease (creatinine >4.0 mg/dl) or severe liver disease (total bilirubin >3 mg/dl, serum glutamic-oxaloacetic transaminase or serum glutamic-pyruvic transaminase >200 units/liter). Subjects with a pacemaker, a history of transient ischemic attack or cerebrovascular accident, or subjects using any drugs known to compete with debrisoquine metabolism (tricyclic antidepressants, monoamine oxidase inhibitors, neuroleptics, phenothiazine, quinidine, amiodarone, flecainide, procainamide, tocainide, or nafcillin) were excluded. Control subjects with a history of any prior malignancy other than excised basal cell carcinoma of the skin, and case subjects with any active malignancy within the prior 5 years or who had already received chemotherapy or radiotherapy for lung cancer were excluded.

Questionnaire and Medical Record Review. An in-person interview requiring approximately 45 min was administered to the subjects by a trained interviewer. Data collected included sociodemographic characteristics, recent and past tobacco use, personal medical history, caffeine and vitamin use within the prior month, alcohol use, family history of cancer and lung disorders, current medications, and lifetime occupational and residence history. Exposure to environmental tobacco smoke was defined as ever living with someone who smoked cigarettes. Case medical records were reviewed to abstract selected information including histological diagnoses from pathology reports, results of clinical and pathological staging, and medications administered. Control medical records were reviewed for current diagnoses, history of medical illness, and current medications.

Debrisoquine Administration. Debrisoquine (Declinax) was obtained from Hoffman-LaRoche Ltd. (Mississauga, Ontario, Canada) under an investigational new drug approval. The subjects received 10 mg debrisoquine p.o. at 10 p.m. and collected the subsequent 8-h urine specimen. The debrisoquine was self-administered by the outpatient subjects, predominantly control subjects from Bethesda, while inpatient subjects received debrisoquine from hospital staff. Subjects discarded the urine voided immediately before taking the debrisoquine and then collected and refrigerated their urine for the next 8 h (including the first morning voided specimen). The overnight protocol was used to minimize inconvenience for subjects as well as hospital staff, and has been shown to be highly correlated with the daytime method (22–23). All urine specimens were returned the morning the collection was completed, and an aliquot was frozen at −20°C that day. Drug administration and adequacy of urine collection was confirmed by calculation of percentage recovery of dose in the urine.

Laboratory Methods. The aliquot of urine was stored at −20°C and later analyzed for measurement of the excreted debrisoquine and principal metabolite 4-hydroxydebrisoquine. Samples were analyzed by HPLC using the method of Hempenius *et al.* (24) using a Hewlett-Packard Model 1090 HPLC (Rockville, MD) equipped with an autosampler/autoinjector and a Hewlett-Packard Model 1046A fluorescence detector as described previously (25–26). Briefly, an excitation wavelength of 194 nm was used while monitoring emission at 572 nm. The separation was carried out on a SPHERI-5 CYANO (5 μm, 100 × 4.6 nm) cartridge column equipped with a 1-cm CYANO guard cartridge (Applied Biosystems, Brownlee columns). The mobile phase was prepared by adding 500 μl of triethylamine (Aldrich Chemical Co.) to approximately 850 ml of water. Phosphoric acid (Fisher Scientific) was used to adjust the pH of the solution to 3.5. HPLC-grade acetonitrile (100 ml; Burdick & Jackson, Muskegon, MI) was added and the solution was brought to 1 liter with water. This solution was applied with HPLC-grade methanol (Burdick & Jackson) in the proportion of 90:10 at a flow rate of 1 ml/min. The resulting mobile phase composition was 81:9:10 aqueous buffer:acetonitrile:meth-

Table 1 Number and percentage distribution of sociodemographic and smoking characteristics of Caucasian subjects used in analysis.

	Bethesda		Quebec		Total	
	Control (n = 135)	Case (n = 109)	Control (n = 238)	Case (n = 226)	Control (n = 373)	Case (n = 335)
Age						
<55	37 (27)	33 (30)	45 (19)	44 (19)	82 (22)	77 (23)
55–64	50 (37)	45 (41)	83 (35)	74 (33)	133 (36)	119 (36)
≥65	48 (36)	31 (29)	110 (46)	108 (48)	158 (42)	139 (41)
Sex						
Male	93 (69)	75 (69)	166 (70)	164 (73)	259 (69)	239 (71)
Female	42 (31)	34 (31)	72 (30)	62 (27)	114 (31)	96 (29)
Education						
<12 yr	3 (2)	10 (9)	180 (76)	188 (83)	183 (49)	198 (59)
12 yr	16 (12)	31 (28)	22 (9)	14 (6)	38 (10)	45 (14)
13–16 yr	47 (35)	38 (35)	23 (10)	17 (8)	70 (19)	55 (16)
>16 yr	69 (51)	30 (28)	13 (5)	7 (3)	82 (22)	37 (11)
Smoking						
Never	58 (43)	6 (6)	49 (21)	5 (2)	107 (29)	11 (3)
Former, <1 ppd[a]	24 (17)	9 (8)	29 (12)	13 (6)	53 (14)	22 (7)
Former, ≥1 ppd	35 (26)	27 (25)	81 (34)	63 (28)	116 (31)	90 (27)
Current, <1 ppd	9 (7)	9 (8)	28 (12)	15 (7)	37 (10)	24 (7)
Current, ≥1 ppd	9 (7)	58 (53)	51 (21)	130 (57)	60 (16)	188 (56)

[a] ppd, packs of cigarettes per day.

anol. All mobile phase components were filtered and degassed through a 0.45-μm Nylon filter (Alltech Associates, Inc.). All samples were analyzed in the same laboratory following procedures described previously (25).

Statistical Methods. The MR was determined by the ratio of proportion dose excreted as debrisoquine to proportion dose excreted as the principal metabolite, 4-hydroxydebrisoquine. The control distribution of natural log of MR was evaluated for each site, as well as all combined, to determine the cut points for classifying phenotypes using a published computerized optimization method (27). The MR cut points used by Ayesh *et al.* (9) (1.0 and 12.6) and by Caporaso *et al.* (27) (1.9 and 20.8) were also applied to these data for comparison of phenotypes. Stratified and multivariate analyses were used to examine the data for effects of confounding and effect modification. ORs and 95% CIs were obtained from logistic regression models performed with the BMDP statistical analysis program (28). Variables examined in the logistic regression modeling included the matching variables (age, sex, and hospital); other variables were also examined as potential confounders. These included education (in tertiles and ≤12 or >12 years), exposure to environmental tobacco smoke (ever/never), reported history of a first-degree relative with any cancer, vitamin use, medication use (any medication which might interact with debrisoquine metabolism, β-blockers, steroids, antibiotics, narcotics, antihypertensives, antiarrhythmics, benzodiazepines, antidepressants, or antipsychotics), control diagnoses, and occupational exposure to asbestos. In addition, smoking was examined by status (never, former, or current), a combination variable (never smokers, former smokers of 20 cigarettes/day or less, former smokers of more than 20 cigarettes/day, current smokers of 20 cigarettes/day or less, current smokers of more than 20 cigarettes/day), pack-years of smoking in quartiles, and examining number of cigarettes smoked/day and pack-years of smoking as continuous variables with smoking status as a covariate. Since OR estimates were similar regardless of which smoking variables were used, smoking status was included in the final models. Former smokers were individuals who had not smoked one year prior to diagnosis for cases and 1 year prior to interview for controls. Pack-years were calculated as one-twentieth the product of the total number of years of smoking and the average number of cigarettes smoked/day. ORs presented are adjusted for age (tertiles), sex, education (≤12 years *versus* >12 years), smoking status (never, former, or current), and hospital.

Results

One hundred twenty-one of 175 (69%) and 230 of 261 (88%) eligible case subjects completed both debrisoquine phenotyping and the interview questionnaire at Bethesda and Quebec, respectively. One hundred thirty-nine of 234 (59%) eligible outpatient control subjects completed both debrisoquine phenotyping and the interview questionnaire at Bethesda, compared to 240 of 245 (98%) of inpatient controls at Quebec.

Three hundred fifty-one patients with untreated histologically confirmed lung cancer completed this study. Since only 13 nonwhite case patients were enrolled, the analysis was limited to Caucasians. Three cases missing debrisoquine metabolism data were excluded from the analysis. Thus, the final analysis was limited to 109 cases from Bethesda and 226 cases from Quebec. Three hundred seventy nine frequency-matched control subjects completed the study. Four nonwhite controls and 2 controls who were missing either the questionnaire or the debrisoquine metabolism data were also excluded, leaving 373 controls. Sociodemographic variables for subjects included in the analysis are presented in Table 1.

The patient populations at the National Naval Medical Center and Laval Hospital differed in several ways. Patients at Quebec tended to be older than those at Bethesda (median age was 64 and 59 years, respectively). Approximately 30% of cases at both sites were women. Patients at

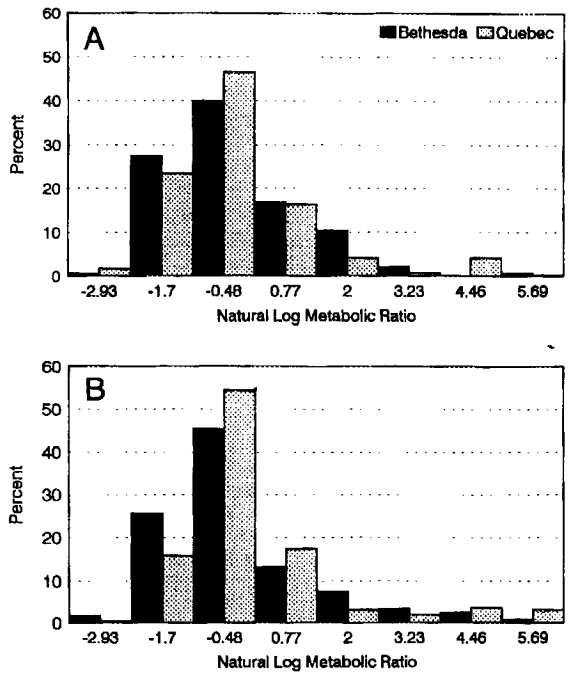

Fig. 1. *A*, distribution of the natural log of the debrisoquine metabolic ratio among control subjects expressed as the percentage of total controls by site. *B*, distribution of the natural log of the debrisoquine metabolic ratio among cases expressed as the percentage of total cases by site.

Bethesda tended to have higher levels of education; the median level was 16 years compared to 7 years at Quebec, and controls had higher levels of education than cases, as shown in Table 1. Smoking patterns also differed, with Bethesda cases smoking a mean of 53 pack-years compared to 65 pack-years by cases at Quebec. Among controls, differences in smoking were even more dramatic with never smokers comprising 43% of Bethesda controls compared to 21% of Quebec controls, and current smokers 13 and 33%, respectively. Control subjects smoked a mean of 18 pack-years at Bethesda compared to 44 pack-years at Quebec. Exposure to environmental tobacco smoke was reported by 74 and 89% of controls at Bethesda and Quebec, respectively. Family history of any cancer in a first-degree relative was reported by 56 and 43% of controls and 45 and 56% of cases at Bethesda and Quebec, respectively.

The percentage distribution of the natural log of the debrisoquine MR is presented in Fig. 1 for controls and cases at each site. The distributions do not differ significantly by site, and the cut point for PM *versus* grouped IM/EM was ln(MR) = 2.0 for both sites, separately and pooled. Using three distributions did not improve the fit for our data, similar to reports by others (16, 27), and thus EM and IM were combined (IM/EM) for further analyses.

Overall, the adjusted estimate of the relative risk of lung cancer for IM/EM compared to PM was 0.6 (95% CI, 0.3–1.2), (Table 2). This OR did not differ by study site. The crude OR was 0.8, and the decrease with adjustment was due to adjusting for smoking. The same results were seen for adjusting for smoking using never, former, and current smokers, as well as using never, light *versus* heavy former and current smokers, or pack-years of smoking.

Since some reports have categorized the distribution into three phenotypes using different cut points, we analyzed our data using the cut points from Ayesh *et al.* (9) and Caporaso *et al.* (27) and demonstrated no increase in risk for EMs, with ORs less than 1.0 for both cut points (Table 3). We further divided the MRs into 5 categories to isolate the very rapid metabolizers with MR <0.2 and to present adjusted ORs in Table 3. Using PMs with MRs of 12.6 or greater as the referent group, no elevated risk was observed in any of the categories.

Table 4 presents risks adjusted for age, sex, education, and hospital according to smoking status and quartiles of pack-years of smoking, with the lowest quartile consisting of never smokers. Lung cancer risk associated with debrisoquine phenotype was similar for never, former, and current smokers, with ORs for IM/EMs being less than 1.0 in each smoking group. When examined by pack-years of smoking, the 31–49 pack-year quartile had an adjusted OR of 2.5 (95% CI, 0.6–10.7) but all other ORs were less than 1.0. Although the adjusted OR for never smokers was the lowest, none of the ORs were significantly different from 1.0 (Table 4). Risk estimates did not differ by sex or hospital.

The composition of histological types and stage differed by study site. At Quebec, 43% of cases had squamous cell carcinoma compared with 14% at Bethesda. Small cell carcinoma and adenocarcinoma each accounted for 34% of cases at Bethesda, compared to 4 and 26%, respectively, at Quebec. Analyses were conducted by histological type of lung cancer, examining squamous cell carcinoma, small cell carcinoma, adenocarcinoma, and all other histologies, and showed no significant difference in risk by histology (Table 5); all ORs for IM/EMs were less than 1.0. Similarly, there was no difference in the OR according to stage (Table 5).

Since the control subjects were recruited from two outpatient surgical clinics at Bethesda, no single medical diagnosis predominated. At Quebec, controls were recruited from inpatients on several services, and 40% carried a diagnosis of COPD in contrast to only 1 of 135 at Bethesda. Six % of controls with COPD were PM compared to 8% of controls without COPD. For all controls, medical diagnoses included COPD (26%), hypertension (24%), arthritis (14%), history of gall bladder disease (11%), diabetes (8%), ulcer disease (6%), pneumonia (6%), thyroid disease (5%), and prior myocardial infarction (4%). The proportion of PM did not vary significantly by diagnosis ($P = 0.7$).

Using our study-based cut point values to characterize PMs *versus* IM/EMs, we found the proportion of PMs to be 7% among controls and 9% among cases. Because our proportion of control PMs was slightly lower than the 8–10% deficient metabolizers reported in some studies of other Caucasian populations, and the proportion of case PMs was higher than observed in some other case-control studies of lung cancer, we examined the influence of medications used 24 h prior to debrisoquine phenotyping and during the previous month. We found that among controls, medication use differed by site, with Quebec controls more likely to be taking antibiotics, corticosteroids, and bronchodilators than controls at Bethesda. In both hospitals, controls took these agents as well as antihypertensives more commonly than cases. Specific medications and categories of medications were examined. Since β-blockers are one class of medication metabolized in part by *CYP2D6* (29), we examined use of β-blockers as a group and of specific β-blockers. There was no excess of PMs among subjects

Table 2 Estimate of relative risk of lung cancer using cut points based on the distribution of the natural log of the metabolic ratio among control subjects.

	Control	Case	Crude OR	Adjusted OR[a]	95% CI
Bethesda					
PM[b]	8	8		1.0	
EM	127	101	0.80	0.69	0.21–2.25
Quebec					
PM	19	21		1.0	
EM	219	205	0.85	0.62	0.30–1.30
Both Sites					
PM	27	29		1.0	
EM	346	306	0.82	0.64	0.34–1.19

[a] Odds ratios adjusted for age, sex, smoking, education, and hospital where appropriate.
[b] PM, poor metabolizers with ln (MR) > 2; EM, extensive metabolizers with ln(MR) ≤ 2.

Table 3 Estimate of relative risk of lung cancer using cut points based on different definitions of phenotype.

	Control	Case	Crude OR	Adjusted OR[a]	95% CI
Ayesh et al. cut points					
PM (MR > 12.6)	25	25		1.0	
IM (MR 1–12.6)	101	84	0.83	0.57	0.28–1.16
EM (MR < 1)	247	226	0.91	0.71	0.37–1.37
Caporaso et al. cut points					
PM (MR > 20.8)	22	23		1.0	
IM (MR 1.93–20.8)	48	42	0.84	0.64	0.29–1.44
EM (MR < 1.93)	303	270	0.85	0.66	0.34–1.31
Five levels of MR					
PM (MR ≥ 12.6)	25	25		1.0	
MR 1.91–12.59	45	40	0.89	0.65	0.29–1.43
MR 1.01–1.9	56	44	0.79	0.55	0.25–1.18
MR 0.21–1.0	214	194	0.91	0.69	0.35–1.34
Very EM (MR ≤0.2)	33	32	0.97	0.97	0.42–2.24

[a] Odds ratios adjusted for age, sex, smoking, education, and hospital.

taking β-blockers compared to nonusers, and use had no effect on the debrisoquine phenotype/lung cancer association. The geometric mean (± SD) of the MR for controls using β-blockers *versus* nonusers was 0.98 ± 4.9 and 0.88 ± 4.9, respectively. These did not differ significantly among either controls ($P = 0.7$) or cases ($P = 0.5$). Exclusion of subjects taking β-blockers also had no effect on the OR. Other categories of medications examined included histamine receptor antagonists, antiarrhythmics, narcotic analgesics, nonnarcotic analgesics, antibiotics, antihypertensives, anxiolytics and hypnotics, corticosteroids, bronchodilators, and vitamins. No effect on the proportion of PMs by medication use was observed, and thus there was no effect on the debrisoquine phenotype/lung cancer association.

Discussion

We found no difference in the distribution of debrisoquine metabolic ratios between lung cancer cases and matched controls, and thus we were unable to confirm the reports of an excess lung cancer risk among extensive metabolizers of debrisoquine (9–12). This is the largest case-control study to examine the association between lung cancer and debrisoquine metabolic phenotype. Our study was designed to have an 80% power to detect an OR of 2 based on 90% of control subjects being extensive metabolizers, as reported by Ayesh *et al.* (9). While the power of the study decreased due to an observed proportion of PMs among controls of 7%, we still had 80% power to detect an OR of 2.7, which would have been adequate if we had observed the 4.6-fold differences reported in other studies (9–10).

In contrast to previous reports, the frequency of PMs in our cases was slightly higher than that of the controls. Our 81% participation rate among cases may have allowed enrollment of more cases who were PMs than other studies have reported. The only other study reporting response rates achieved participation from only 33% eligible cases (10). PMs may be less willing to participate in studies of drug metabolism if they have experienced previous adverse drug reactions related to deficient metabolizing capability, and thus participation by PMs may vary with recruitment methods. We also observed a slightly lower frequency of PMs among our controls than those reported in some Caucasian populations (3), but similar to others (7–8). This variation in reported frequency of PMs among noncancer patients may be related to the different populations reported, ranging from healthy volunteers to patients with COPD.

Because we defined metabolic phenotypes based on the observed distribution in our control population we used cut points different from other studies. Our results were not affected by examining our data using other published cut points. We further subdivided the distribution of MR into five levels, since some investigators have hypothesized that the very rapid metabolizers represent the homozygous extensive metabolizers and have the greatest increase in risk (10). Using PMs as the referent category no subgroup demonstrated an increased risk.

The distribution of lung cancer histological types and stage of disease reflects the referral patterns and treatment options available at the respective institutions. Specifically, Bethesda had active treatment studies for small cell lung cancer and Phase I/II studies for non-small cell lung cancer during the study period, while Laval Hospital primarily offered surgical resection and rarely offered radiotherapy or chemotherapy. Some investigators have reported that patients with adenocarcinoma were more likely to be PMs than patients with squamous cell or small cell histologies (9–11), suggesting that the increase in risk with extensive debrisoquine metabolism occurred only in smoking-related histologies. Our study and others have not found a difference in risk by histology (16–18).

Different studies have varied in methods to conduct metabolic phenotyping using different doses of debrisoquine, different durations of urine collections, different as-

Table 4 Distribution of cases and controls according to smoking status and metabolic phenotype with stratified odds ratios.

	PM[a]		EM		Crude OR	Adjusted OR[b]	95% CI
	Controls	Cases	Controls	Cases			
Nonsmokers	9	2	98	9	0.41	0.23	0.03–1.66
Ex-smokers	15	12	154	100	0.81	0.84	0.37–1.88
Current smokers	3	15	94	197	0.42	0.42	0.12–1.51
Ever-smokers by pack-years[c]							
1–30 PY	6	7	90	39	0.37	0.40	0.12–1.37
31–49 PY	6	4	62	72	1.74	2.5	0.58–10.7
50+ PY	6	16	96	186	0.73	0.66	0.25–1.79

[a] PM, poor metabolizers with ln(MR) >2; EM, extensive metabolizers with ln(MR) ≤2.
[b] Odds ratios adjusted for age, sex, education, and hospital.
[c] PY, pack-years, one-twentieth the product of the total number of years of smoking and the average number of cigarettes smoked per day.

Table 5 Estimated risk of lung cancer for extensive metabolizers by histology and stage

	PM[a]	EM	Total	Adjust OR[b]	95% CI
Controls	27	346	373	1.0[c]	
Histology					
Squamous cell carcinoma	8	105	113 (34%)	0.8	0.32–1.96
Small cell carcinoma	4	43	47 (14%)	0.3	0.09–1.08
Adenocarcinoma	12	91	96 (31%)	0.4	0.19–0.97
Other non-small cell	5	67	72 (21%)	0.7	0.25–2.12
Stage[d]					
I/II	12	118	130 (51%)	0.6	0.27–1.32
IIIA/IIIB/IV	13	143	156 (47%)	0.6	0.29–1.35

[a] PM, poor metabolizers with ln(MR) >2; EM, extensive metabolizers with ln(MR) ≤2.
[b] Odds ratios adjusted for age, sex, smoking, education, and hospital.
[c] Referent category.
[d] Does not include one patient with Stage 0, one with mesothelioma, or patients with small-cell lung cancer.

say techniques, different times of day for the phenotyping and prevalent cases. Speirs *et al.* (18) and Roots *et al.* (16) maintain that debrisoquine doses lower than 10 mg yield equivalent results, but most data, including ours, has been collected using the 10-mg dose. Since the MR is a ratio of the proportion of dose excreted as debrisoquine to its metabolite 4-hydroxydebrisoquine the ratio is sensitive to small changes in the concentration of the metabolite. The concern is that small doses may result in underestimates of the metabolizing capability if the metabolite is present in very small concentration. Likewise, urine collection for less than 8 h may not yield an accurate representation of metabolizing capability. In light of the different assay techniques available, we incorporated an analysis of laboratory reproducibility into our study design. We found that electron capture GC had excess variability compared to HPLC, and that HPLC also had greater sensitivity (25). We have also demonstrated previously a high correlation of MR from daytime and overnight phenotyping (22). One positive (11) and one negative (17) study also used overnight phenotyping. It is unlikely that overnight phenotyping, when used for both cases and controls as in our study, would confound any effect of metabolizing capability on lung cancer risk. Our study design also attempted to address potential problems with the use of prevalent cases in other studies as well as the potential for induction of debrisoquine metabolism by lung tumors. We demonstrated previously that metabolic phenotype did not change following surgical resection of lung tumors in 104 patients (26), suggesting that the tumor does not induce metabolism and that prevalent cases are likely to have had the same metabolic phenotype at diagnosis.

Several medications are metabolized by *CYP2D6,* many of which act as competitive inhibitors to debrisoquine metabolism (29–30). More frequent use of these medications by cases compared to use by controls could have falsely increased the number of PMs among cases. To circumvent this we excluded from the analysis all subjects taking medications known to affect debrisoquine phenotype or alter debrisoquine metabolism. Other medications were examined for their possible influence on debrisoquine metabolism, and the proportion of PMs did not differ significantly between users and nonusers. The lack of an association between metabolic phenotype and lung cancer risk was unchanged when we excluded subjects concurrently using β-blockers, narcotic analgesics, corticosteroids, antibiotics, bronchodilators, nonexcluded antiarrhythmics, nonnarcotic analgesics, antihypertensives, or anxiolytics and hypnotics.

Some investigators have used only smokers as control subjects (9–12, 15) and hypothesized that increased debrisoquine metabolism is a risk factor only in smokers. These studies have been unable to address confounding of the association by smoking. In contrast to Caporaso *et al.* (10), we found that adjusting for smoking lowered the OR. We found no elevated risk among current or former smokers. A nonsignificant elevated risk was observed among those with moderate pack-years of smoking, but ORs for light and heavy smokers were less than 1.0. There is still insufficient data to support a clear-cut biological mechanism for an association between debrisoquine metabolism and lung cancer risk, despite some evidence that *CYP2D6* may activate *NNK* (20) or metabolize nicotine (21).

The evidence linking lung cancer to the debrisoquine metabolic phenotype is equivocal, with several studies in addition to ours finding no excess risk for IM/EM (15–18) and others reporting an excess number of extensive metabolizers among lung cancer cases (9–12). We included only untreated patients, but we also demonstrated that surgically resected patients retained the same metabolic phenotype before and after surgical resection (26). Similar to others, we excluded subjects taking drugs potentially metabolized by *CYP2D6,* but uniquely, we applied these exclusion criteria to control subjects as well. We also examined drugs taken 24 h prior to phenotyping as well as

in the month prior, since any changes in endogenous metabolism induced by medication may not reverse in 24 h. Some investigators have applied different exclusion criteria to controls, requiring controls to be taking no medications and excluding potentially competing drugs for cases only. This difference could potentially introduce bias in the case-control comparison. In addition, we carefully assessed the distribution of PM *versus* IM/EM by medication use as well as by control diagnoses to attempt to explain any confounding factors in our study to explain the lack of an association between lung cancer risk and debrisoquine metabolism.

By identifying control subjects from the same population from which the cases were drawn and by matching age, race, sex, and hospital we obtained a population very similar to the cases. The controls had a variety of medical diagnoses but no predominant one and examination of phenotype by diagnosis demonstrated no association.

Our study was able to address noncancer patients with COPD separately from matched controls without COPD. The frequency of PMs among controls with COPD did not differ significantly from controls without COPD, and thus they were felt to be comparable to the other control subjects. Potential differences between COPD controls and the non-COPD controls with regard to debrisoquine metabolizing capability do not explain the different findings in studies using these controls (9–10).

One weakness of our study was the 59% response rate among control subjects at Bethesda. The targeted outpatient population was scheduled for clinic visits, and some patients did not have the additional time to spend participating in the study, while others had their clinic appointments cancelled and were unable or unwilling to schedule a visit solely for our study. It is possible that if PMs had previously experienced an adverse drug reaction they would be more likely to refuse participation, and this could result in underrepresentation of PMs among our Bethesda controls. This may in part explain why there were fewer PMs among controls in Bethesda compared to Quebec, although the difference was not significant.

The molecular defect resulting in deficient debrisoquine metabolism has been progressively elucidated, particularly with the advent of PCR technology (31–33). While the presence of variant alleles is correlated with debrisoquine metabolic phenotype, there is much overlap in the distribution of the MR by genotype (34). Consequently, metabolic and genetic categories do not correspond precisely. Until the metabolic consequences of the numerous mutations are clarified, metabolic phenotyping will be useful in studies of the debrisoquine polymorphism.

In summary, in the largest case-control study of debrisoquine metabolism and lung cancer, we failed to demonstrate any excess risk of lung cancer among extensive metabolizers of debrisoquine. These findings do not support the use of debrisoquine metabolic phenotype as a marker for lung cancer susceptibility.

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